

# Organoleptic Properties and Gas Chromatography Patterns of Steam Distillates from Fresh and Stale Milk Fat 3012

A. TAMSMA, F. E. KURTZ, A. KONTSON, and M. J. PALLANSCH

Dairy Products Laboratory  
Eastern Utilization Research and Development Division, USDA  
Washington, D.C.

## Abstract

We have examined the chromatographic patterns as well as the flavor characteristics of steam distillates of milk fat to obtain a more objective procedure than that given solely by taste panel evaluation for following the development of stale flavor during the storage of fat-containing dairy products. Both fresh and stale milk fats were steam deodorized at 50 and 75 C at 1 mm. Stale milk fat was also steam deodorized at 100 (1 mm) and at 50 C (3 mm).

One-millimeter pressure is preferable to higher pressures. Distillation at 75 C or higher produces artifacts, volatile compounds generated from precursors, undesirable for following the formation of stale-flavor compounds during storage. Volatiles are recovered less completely at 50 C than at higher temperatures, but artifact generation is low. Moreover, the flavor of volatiles recovered at 50 C is characteristic of stale fat, and the chromatographic patterns of the 11 most prominent peaks correlate well with the flavor intensity and with the expected relative concentrations of the pertinent off-flavor compounds produced by storage of fats at various temperatures.

The elimination of oxygen from the environment of milk fat or milk fat-containing materials does not prevent flavor deterioration during storage, especially at elevated temperatures (5). The flavor developed under nonoxidative conditions, though simply termed stale, obviously results from complex chemical reactions within the fat (2) that tend to elicit a wide variety of responses from skilled dairy product judges.

Since our studies, oriented toward the development of a flavor-stable whole milk powder, were seriously hindered by this complexity of judge response in the taste panel operations necessary to quantify the staling rate of ex-

perimental samples, we sought more objective methods for determining the extent of stale flavor in fat-containing dairy products.

Steam distillation has been widely used for removing volatile flavor compounds from fats and oils for subsequent characterization by gas chromatography. At sufficiently high temperatures, certain precursor compounds in milk fat are completely converted to their off-flavored derivatives (1). At sufficiently low temperatures these reactions can be avoided or minimized, but the off-flavor compounds in the fat are incompletely removed. Nevertheless, due to the inherent simplicity of steam distillation and gas chromatography procedures, we decided to study their applicability to the problem of objectively determining the extent of stale flavor development in experimental products.

We describe procedures which permit the isolation of the off-flavored compounds from stale milk fat in amounts correlated with their concentrations in the fat. Data pertinent to the selection of these procedures also describe the relationships between organoleptic responses to the steam distillates and the principal peak heights of their gas chromatograms.

## Materials and Methods<sup>1</sup>

Milk fat was prepared by melting down butter churned from fresh pasteurized cream and packed in an inert atmosphere as described (5). Samples were stored at -10, 4, and 27 C.

Samples of fresh and stored milk fat were steam distilled in an apparatus similar to that described by Riemenschneider et al. (4), but so modified by increasing the bore of the vacuum pumping and trapping system that pressures of 1 mm Hg, read through a special outlet directly over the fat surface, could be maintained. Distillations were carried out by flowing steam through 275-g samples of milk fat at a rate equivalent to 50 ml water per hour.

The water and organic materials caught in

<sup>1</sup> Mention of brand or firm name does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

the dry-ice plus acetone-cooled trap were melted and used either directly in organoleptic work or extracted to provide samples suitable for analysis by gas-liquid chromatography.

The aqueous distillate was extracted successively with 10-, 5-, 5- and 5-ml portions of methylene chloride. Each extraction was carried out by mechanically shaking for 10 min using a separatory funnel. The combined extracts were concentrated to approximately 1 ml by distilling off the solvent through a 21-cm Vigreux column. After transferring to a 2-ml sample tube the volume of the sample was further reduced by gentle heating to 20  $\mu$ liters. Accurately measured aliquots of approximately 2  $\mu$ liters were used for gas chromatography.

To eliminate contamination of distillates and extracts with materials other than those derived from the milk fat sample under investigation, all glassware was rigorously cleaned. This was done by first washing with detergent and tap water, then rinsing with 12% hydrochloric acid, tap water, and doubly distilled water in this sequence. After this, the glassware was heated in a 125-C oven for at least eight hours before use. The water was doubly distilled in a Heraeus quartz still. The reagent-grade methylene chloride was further purified by distillation through a 20-plate Oldershaw column.

Chromatographic analyses were carried out with a Mikrotek 2000-R gas chromatograph equipped with flame ionization detector and dual 305- by 0.32-cm columns packed with 10% Apiezon L on 80-100 mesh Chromosorb W. With 20 ml/minute nitrogen flowing through the columns, analysis was carried out using temperatures programmed for a 2-min initial hold at 70 C, followed by a 7.5 C/minute rise to 220 C, where it was held until complete.

The organoleptic properties of milk fat and steam distillates were evaluated using a ten-man taste panel of skilled judges and a ten-point score card as described (3). The fats were homogenized into fresh pasteurized skim-milk to produce 3.3% fat recombined milk. Aqueous steam distillates were added to fresh, pasteurized whole milk concentrates, along with sufficient distilled water to reduce total solids content to its original percentage. The amount of distillate used contained the volatiles from stale fat equivalent to, or a whole number multiple of, the fat in the fresh milk concentrate. After preparation, the experimental samples were allowed to equilibrate overnight in a refrigerator before tasting.

## Results

Preliminary chromatographic studies of organic materials isolated from milk fat samples showed that patterns containing at least 50 distinguishable peaks could easily be obtained in reproducible fashion. Analysis of blank samples demonstrated that none of them were artifacts arising from contaminated equipment or solvents.

For study purposes, the 28 largest peaks were arbitrarily chosen and identified by number in order of their appearance on the chromatogram. The patterns of the 11 most prominent peaks in this group were studied in detail and are the subject of this paper.

Further preliminary studies indicated that the rate of total organic material removal from fat samples was an inverse function of the pressure over the fat surface. During distillation periods of similar length, approximately 2.7 times more material was recovered when the internal pressure of the still was dropped from 3 to 1 mm Hg. To minimize analysis time and artifact formation, all subsequent work reported here was carried out at the 1-mm pressure obtainable in our modified equipment.

The temperature at which steam distillation of the fat is carried out is also an important consideration. In Fig. 1 we see the change in peak heights of the 11 largest peaks in the chromatographic patterns of materials isolated from fresh milk fat by steam distillation. These and all other data have been adjusted to account for dilution factors in such fashion that each pattern represents volatiles recovered from 11 g of milk fat. Therefore, direct comparison of peak heights shows an increase in material represented by each peak as the temperature of distillation increases. Since the materials of Peaks 13 and lower are readily distilled at 50 C (Fig. 2 and 3), these increases indicate generation of artifacts from precursors at the higher temperature. Illustrated by Fig. 1 also is the fact that isolation of materials having volatilities greater than that of the material forming Peak 13 is relatively complete after three hours, even if distillation is carried out at 50 C.

Evidence that steam distillation at 50 C is adequate for removing volatiles formed from precursors in milk fat during storage is found in Fig. 2. Here, peak heights of the patterns of the volatiles isolated from stale milk fat are shown. When compared with patterns shown in Fig. 1, they show an over-all increase in materials isolatable from milk fat due to staling, regardless of temperature used in the distillation.

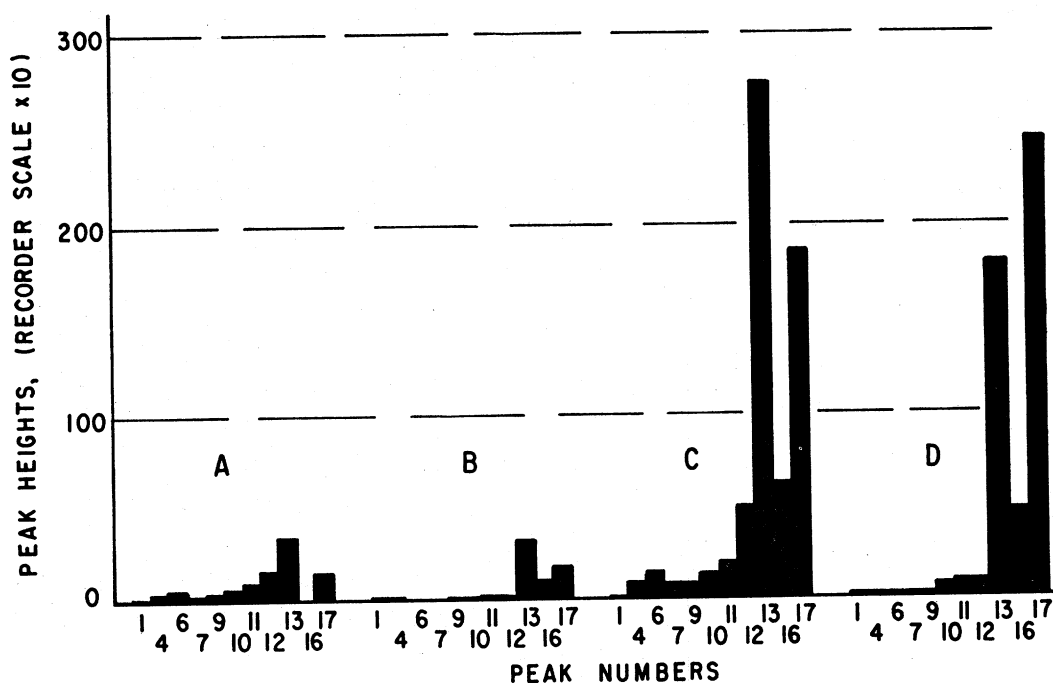


Fig. 1. Chromatographic peak heights of volatiles obtained from fresh milk fat by steam deodorization at 1-mm pressure.

- A. 50 C, first deodorization, three hours.
- B. 50 C, second deodorization, three hours.
- C. 75 C, first deodorization, three hours.
- D. 75 C, second deodorization, three hours.

Further removal of less volatile materials by elevated temperatures is indicated by peak height increase with increased isolation temperature.

Fig. 3 shows that the materials of Peak 13 and lower are recovered in good yield by distillation at 50 C, the less volatile materials in poorer yield. These data, along with those on fresh fat (Fig. 1), are evidence that increasing the distillation temperature to 75 C markedly increases formation of artifacts. The primary precursors of stale flavor compounds and, consequently, the artifacts derived from these precursors during steam deodorization are, of necessity, at a maximum in fresh fat. Comparing Fig. 1 and 2, it is evident that even if all of the materials obtained from the fresh fat were generated by the deodorization procedure at 50 C (an assumption which our data indicate is not true), the artifacts would be only a low-level background for the compounds formed during storage.

During the chromatographic study of materials in steam distillates taken from fresh and stale milk fat samples, an interesting effect was observed. Even though the concentration of

volatiles in milk fat could be reduced to very low levels by steam distillation, on standing overnight additional volatiles were generated. This occurred in spite of storage of the fat under nitrogen and at 0 C. An example of this phenomenon is shown in Fig. 3.

From the data presented we concluded that changes in milk fat during storage could be followed by studying the chromatographic patterns of materials isolated from the samples by steam distillation at 50 C and 1-mm pressure. This is substantiated by data of the type shown in Fig. 4. Here the heights of the principal peaks in chromatographic patterns obtained from an analysis of volatiles in fats stored seven months at various temperatures are shown to increase with increasing storage temperature.

The manner in which the material in the distillates relates to flavor and the organoleptic properties of the milk fat after being subjected to steam distillation at various temperatures is shown in Table 1, which can be correlated with Fig. 2.

In all cases, the distillates added back to fresh milk reproduced qualitatively the flavor characteristics of the stale fat. Steam distilla-

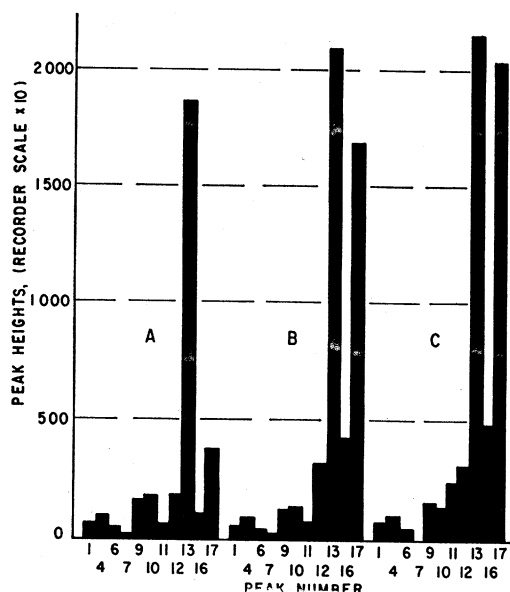


Fig. 2. Chromatographic peak heights of volatiles obtained from stale milk fat by steam deodorization at various temperatures. All samples had been stored at 27 C and deodorized six hours at 1-mm pressure.

- A. Stored ten months and deodorized at 50 C.  
B. Stored 12 months and deodorized at 75 C.  
C. Stored 12 months and deodorized at 100 C.

tion at 50 C for six hours did remove flavor constituents, but not completely. These constituents were more effectively removed from the fat by distillation at higher temperatures.

In a practical sense, the isolation of off-flavor constituents from stale milk fat by steam distil-

lation at 50 C cannot be significantly improved by extending the distillation time. This is shown by the data in Table 2, which can be correlated with Fig. 3. From this it can be seen that after nine hours of distillation the concentration of materials in the distillate is so low that even when using a fivefold increase in the normal volume of distillate to reconstitute concentrated whole milk to normal total solids content, insignificant changes in flavor score were observed.

#### Discussion

Volatiles can be removed from stale milk fat and added back to fresh whole milk concentrate to produce milk with the same qualitative organoleptic characteristics as those of a product made by homogenizing the stale fat with fresh skim milk.

Distillation at 75 C or higher produces artifacts undesirable for following the formation of stale-flavor compounds during storage. The recovery of volatiles by distillation at 50 C and 1-mm Hg pressure is less complete than at the higher temperature, but artifact generation is very low. Moreover, their flavor is still characteristic and their chromatographic patterns correlate well, both with flavor intensity and the expected relative concentrations of the pertinent compounds in stale fats produced by storage at various temperatures.

The use of pressures, measured over the fat, higher than 1 mm offers no discernible advantage. To recover the same amount of volatiles, higher pressures would extend the time

TABLE 1. Effect of deodorization temperature on removal of off flavors from stale milk fat.<sup>a</sup>

Deodorization temperature	Sample	Flavor score		No. of major flavor criticisms <sup>b</sup>			
		Original	Treatment effects	Stale	Lactone	Unclean	Foreign
(C)							
50	Stale fat	34.9		3.3	3.3	1.7	0.0
	Deodorized fat		+1.7	2.8	0.0	0.6	0.6
	Market milk	36.8		1.1	0.0	0.6	0.0
	Market milk + volatiles		−1.1	3.3	1.7	1.7	0.6
75	Stale fat	34.5		2.0	3.5	2.5	1.0
	Deodorized fat		+2.3	2.0	0.0	1.5	0.5
	Market milk	36.7		0.0	0.0	3.0	0.5
	Market milk + volatiles		−2.1	2.5	5.0	1.0	0.5
100	Stale fat	34.6		3.3	3.3	1.7	1.1
	Deodorized fat		+2.1	0.6	0.0	2.8	0.0
	Market milk	36.7		0.0	0.0	3.9	0.6
	Market milk + volatiles		−2.0	3.3	2.8	1.1	1.1

<sup>a</sup> Milk fat stored at 27 C for 11 to 13 months. All deodorizations were for six hours at 1-mm pressure.

<sup>b</sup> The 50-, 75-, and 100-C experiments were judged by, respectively, nine-, ten-, and nine-man panels. The numbers of criticisms shown above for the nine-man panels were obtained by dividing the reported figures by 0.9.

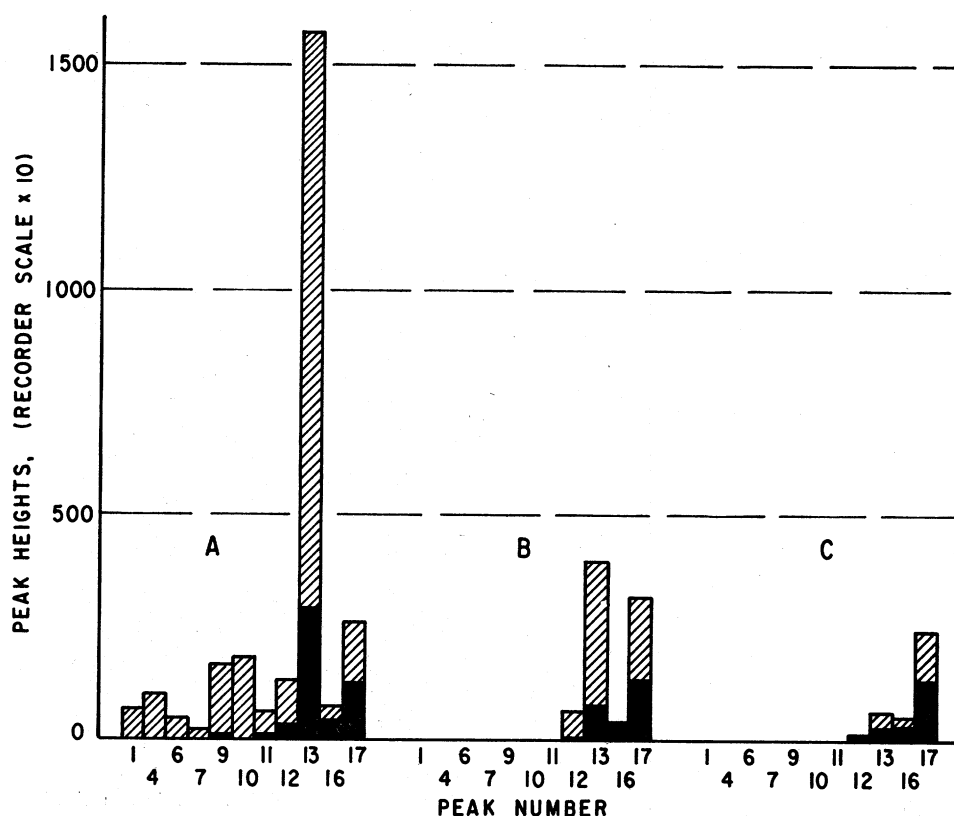


Fig. 3. Chromatographic peak heights of volatiles obtained from stale milk fat by repeated steam deodorizations. The sample had been stored at 27 C for ten months and treated by six successive deodorizations, each for three hours at 50 C and 1-mm pressure.

A. Hatched area, first day, first deodorization; dark area, first day, second deodorization.

B. Hatched area, second day, first deodorization; dark area, second day, second deodorization.

C. Hatched area, third day, first deodorization; dark area, third day, second deodorization.

of analysis and increase the formation of artifacts.

Even though the heights of the peaks in the

chromatograph patterns we have selected for study are indicative of the extent of flavor deteriorations in butteroils stored under non-

TABLE 2. Effect of repeated deodorizations at 50 C on flavor of volatiles removed from stale milk fat.<sup>a</sup>

Deodorization period	Flavor score of control	Concentration of added volatiles	Effect of volatiles on flavor score	No. of the major criticisms <sup>b</sup>			
				Stale	Lactone	Unclean	Foreign
First day	36.9	Double	-1.9	1.5	2.5	4.5	1.0
First three hours		Quintuple	-3.4	1.0	1.5	5.0	2.5
Second day	36.9	Double	-0.6	2.0	1.0	2.5	0.0
Second three hours		Quintuple	-1.0	1.0	3.5	1.5	0.0
Third day	36.7	Double	+0.2	1.1	0.6	0.6	0.6
Third three hours		Quintuple	-1.2	2.2	4.4	1.7	0.0
Fourth day	36.7	Double	+0.2	0.6	1.7	0.6	0.0
Fourth three hours		Quintuple	0.0	1.7	1.1	1.7	0.6
Fifth day	36.7	Double	0.0	0.0	0.5	1.5	0.0
Fifth three hours		Quintuple	-0.4	1.0	2.0	1.5	0.5
Sixth day	36.7	Double	+0.2	0.5	0.5	0.5	0.5
Sixth three hours		Quintuple	-0.5	0.5	2.0	2.0	1.0

<sup>a</sup> Fat stored ten months at 27 C. Deodorizations at 1-mm pressure.

<sup>b</sup> The number of each criticism shown for the second day was obtained by dividing the number reported by the nine-man panel by 0.9.

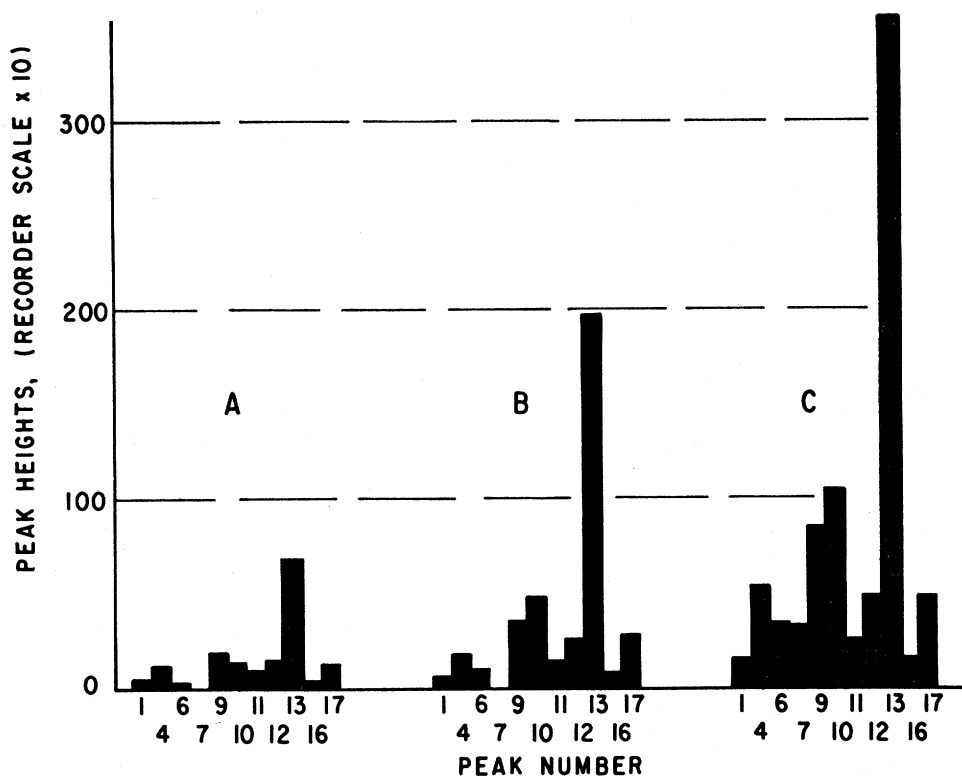


FIG. 4. Effect of storage temperature on chromatographic peak heights of volatiles obtained from milk fat by steam deodorization at 50 C and 3-mm pressure during 2.5 hours.  
 A. Stored seven months at  $-10^{\circ}\text{C}$ .  
 B. Stored seven months at  $4^{\circ}\text{C}$ .  
 C. Stored seven months at  $27^{\circ}\text{C}$ .

oxidative conditions, it should not be inferred from our work that the compounds responsible for stale flavor development in milk fat are necessarily associated with the exact peaks described in this paper. It must be remembered that at least 39 other minor peaks exist in the chromatograms, any one of which may vary directly with the peaks described here.

We have made no attempt to identify the compounds responsible for the peaks in our chromatograms, because we have aimed primarily at devising a fingerprinting procedure capable of objectively characterizing the rate of stale flavor development in milk fat or the fat phase of lipid-containing dehydrated dairy products stored in inert atmospheres, particularly dried whole milk.

At present we are developing methods to isolate, in pure form, the major components in the volatile fraction reported here and determine which one (or more of them) is responsible for the observed stale flavor. It may be

that the concentration changes of the major constituents in this fraction merely coincide with fluctuations of the true off-flavor-producing compounds found therein.

#### References

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